Saskatchewan Agriculture, Food and Rural Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20000051

EVALUATE PLANT PATHOGENS FROM SITE OF WEED ORIGIN FOR BIOLOGICAL CONTROL OF SCENTLESS CHAMOMILE IN SASKATCHEWAN

Funded by: The Agriculture Development Fund

May, 2005

Prepared by: Receiver General for Canada

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Research Branch

FINAL REPORT to ADF
Project 20000051

August, 2003

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AGRICULTURE DEVELOPMENT FUND
PROVINCE OF SASKATCHEWAN

Evaluate Plant Pathogens from the Area of Weed Origin for Biological Control of Scentless Chamomile in Saskatchewan

Gary Peng and Karen L. Bailey

Bioproducts and Bioprocessing
Environmental Health
107 Science Place, Saskatoon, SK S7N 0X2

Hariet L. Hinz
CABI Bioscience Switzerland Center
CAB International
1 Rue des Grillons, Ch-2800 Delémont, Switzerland

Abstract

A project was initiated in 2001 to survey and evaluate naturally occurring microorganisms in Europe to enhance our research on biocontrol of scentless chamomile in Saskatchewan. Field surveys were conducted in three target areas covering southern Germany/eastern France (the Rhine Valley area), eastern Austria and southern UK. Leaf spot was the most common disease symptom observed, especially in the Rhine Valley. A total of 201 fungal isolates (138 in 2001 and 71 in 2002) were obtained and evaluated for biocontrol of scentless chamomile. A group of *Colletotrichum* sp. exhibited moderate weed control and strong host specificity, therefore were subjected to further assessments. These *Colletotrichum* isolates appeared to be close to *C. truncatum*, but the species designation was tentative due to morphological similarities of several species. However, these isolates were considered to be the same as those *Colletotrichum* strains found in Saskatchewan, and none of them showed pathogenicity on any of the field crops tested including lentil. These isolates were efficacious on scentless chamomile ranging from 6-leaf to 13-leaf growth stages. At 20°C they generally required a leaf-wetness period longer than 16 h for maximal infection on scentless chamomile. In comparison to leading indigenous *Colletotrichum* sp. strains, these foreign isolates exhibited a comparable level of weed control, crops safety, and environmental requirement for infection.

Due to additional regulatory requirements for foreign pathogens, we recommend to focus on indigenous strains for further development of biocontrol on scentless chamomile.

Executive Summary

Scentless chamomile is a noxious weed in Saskatchewan. It can spread rapidly due to its prolific seed production and versatile life cycle. Heavy infestations have been reported in several major crop areas in the province, especially in parkland regions of Saskatchewan and Alberta. In research plots, yield losses up to 80% have been observed in cereals. Scentless chamomile is also a difficult weed to control using conventional strategies mainly because herbicides are ineffective once the weed reaches the 4-leaf stage.

Biological control using plant pathogens represents a potential new approach. In 2000, a project was initiated to survey and evaluate indigenous microorganisms for potential inundative biocontrol of scentless chamomile and a large number of microbial isolates were collected from 39 chamomile-infested RMs in the province. The research revealed several promising strains of *Colletotrichum* sp. that caused significant damage to scentless chamomile while proved to be safe to a wide range of crops in the province.

Development of biocontrol agents is a major undertaking that requires substantial resources. Therefore, it would be prudent to explore the biodiversity for the nature's best prior to a development decision. Scentless chamomile is a weed of European origin. In the literature, a broad group of pathogens have been found in association with scentless chamomile or closely related species in Europe, with 32 fungal species recorded (Appendix 1). Although many of the pathogens found are plurivorous, several species including *C. gloeosporioides, Ascochyta matricariae, Fusarium oxysporum,* and *Ramularia matricariae* can be host specific, infecting only a narrow range of plant species.

A project was initiated in 2001, with partial financial support from ADF and collaboration between AAFC Saskatoon Research Center and CABI Bioscience Switzerland Center, to survey and evaluate plant pathogens of scentless chamomile from Europe. Field collections were conducted in three geographic areas covering the Rhine Valley, eastern Austria, and

southern UK. A total of 209 fungal isolates were obtained and evaluated for biocontrol of scentless chamomile. Most organisms showed little effect, but a group of *Colletotrichum* sp. demonstrated moderate weed control in greenhouse conditions. In further assessments, these isolates exhibited strong host specificity, causing severe damage only to scentless chamomile or German chamomile, but not to a range of crops including cereals, oilseeds, pulses, and forages. At 20°C these foreign isolates required up to 16 h of leaf wetness for maximal infection, but this need for a relatively long dew period can be improved with formulation technologies. The foreign isolates were considered to be the same as those of *Colletotrichum* sp. strains found in Saskatchewan, and their taxonomic designation was tentatively given as *Colletotrichum truncatum*.

With a comparable level of weed control and similar biological traits to indigenous strains, the foreign isolates of *Colletotrichum truncatum* were not recommended for further development due to potential regulatory hurdles. However, it needs to be recognized that the survey in Europe was carried out for only two seasons, and several reported host-specific pathogens were not yet found and evaluated. The project was completed according to original objectives and within the budget.

Technical Report

Background and Objectives

Scentless chamomile, *Matricaria perforata* Mérat (Rauschert 1974), is a noxious weed in the Canadian prairie provinces and a Class-3 secondary noxious weed under the Canada Seeds Act (Woo et al. 1991). The native distribution of scentless chamomile ranges from northern Spain, northern Italy and the central Balkan peninsula northwards to most of Scandinavia, eastwards into European Russia and the Caucasus, and parts of western Siberia and central Asia (Kay 1994). It was introduced into North America around the end of the 19th century from Europe (Woo et al. 1991), and has been a very adaptable species which can grow either as a winter or summer annual, a biennial or a short-lived perennial (Hegi 1987, Woo et al. 1991).

The scientific nomenclature of scentless chamomile is inconsistent in the literature. Wagenitz (1987) suggested the name *Tripleurospermum perforatum* (Mérat) Laínz. In this report, the name *Matricaria perforata* is used due to the fact that it has appeared most commonly in recent publications. In Canada, it occurs in all provinces, but is most abundant in the Prairie Provinces and the Atlantic region. These two centers of abundance correspond to the distribution of two different biotypes; diploid and tetraploid. The diploid type occurs mostly in the Atlantic region and the tetraploid type on the prairie (Woo et al. 1991). In comparison, the tetraploid biotype developed into a much more serious weed problem on disturbed or cultivated land of the Prairies, where it has potential to cause considerable yield losses in various crops (Cole 1994, Douglas et al. 1991, 1992). According to the 1995 Saskatchewan Weed Survey, infestation was widespread in the province, especially in the parkland area. The frequency of scentless chamomile in farmer's fields had increased 4-fold since 1986. In the United States, the weed has been reported from most states north of latitude 37°N, as well as Alabama and Florida (*http://plants.usda.gov/plants/plntmenu.html*).

Scentless chamomile can spread rapidly due to its prolific seed production and versatile life cycle. It occurs in a wide variety of habitats, including annual and perennial crops, pastures, wasteland, roadsides and ditches, urban and industrial areas (Woo et al. 1991). In agricultural areas it is more noticeably common around slough margins, in field depressions and along transition areas such as fence lines and right-of-ways (Bowes et al. 1994). Scentless chamomile is a difficult weed to control in crop fields because established plants are highly tolerant to most post-emergent herbicides. Conventional measures are generally inadequate. Cultural methods including tillage, mowing, and hand pulling have been suggested as general options (Cole 1994). Tillage is only partially effective, especially under moist soil conditions or in heavy-soiled fields, because the fibrous roots of scentless chamomile often adhere to large soil clumps and tillage often merely transplants the weed (Peschken et al. 1990). In addition, in most areas on the prairie tillage is being reduced or eliminated for soil and water conservation. Mowing can reduce seed set but effective timing is a challenge. Often new shoots can emerge and produce flowers and seeds if temperature and moisture conditions are favorable in late summer and early fall. Hand pulling is used by some organic growers or in newly-infested areas, but is apparently impractical for large-operations.

A number of herbicides have been suggested to control scentless chamomile in cereals, but options are lacking for many other crops including pulses and forages (SAF 2002). Of the nine herbicides registered for chamomile control in wheat in Alberta (Ali 1995), five were bromoxynil products that are effective only on young seedlings, and plants that passed the 4-leaf growth stage generally can not be controlled effectively at crop-tolerant rates (Brenzil, SFA, personal communication). The remaining herbicides were either marginally effective, restrictive on subsequent crops, or too expensive. In infested fields, scentless chamomile often becomes the worst weed problem, causing 30-80% of yield reduction in spring wheat (Douglas et al. 1991, Hoag et. al. 1980). In general, over-wintered scentless chamomile is more competitive to spring-seeded crops, and crop yield losses are greater in moist years due to excessive size of the weed. Despite the problem faced by many sectors including producers, rural municipalities, and highways/railroads, the chemical companies have yet put this weed on their priority list for product registration due to a relatively small market potential. Therefore, different strategies are required for more effective management of scentless chamomile.

Biological control using introduced insects has been explored since early 1990's (Peschken and Sawchyn 1993). A seed weevil (*Omphalapion hookeri*) and gall midge (*Rhopalomyia* sp.) have been released in Alberta and Saskatchewan in the past few years to suppress weed populations. Although these insects established in many release sites (Bowes, SFA, personal communications), the short-term impact on the weed is not yet significant. Biological control using plant pathogens (microbes) represents another potential new component in an integrated weed-management strategy. Starting in 2000, a project was initiated at AAFC Saskatoon to evaluate indigenous microbes for inundative biocontrol of scentless chamomile. Extensive field surveys were carried out in Saskatchewan during 2000 and 2001, and a large number of microbial isolates were collected from 39 rural municipalities across the province. A process was employed to evaluate various microorganisms for weed control efficacy and crop safety, and a group of isolates belonging to fungal genus *Colletotrichum* were found to be promising biocontrol candidates.

Developing microbial agents further as potential products is a costly process involving increased investment in studies of mass production and formulation, human health/safety impact, and environmental assessment. Before a development decision, it is prudent to ponder if we acquired "nature's best". For inundative biocontrol, it is judicious to focus on indigenous organisms that are generally perceived to be of lower risk in comparison to exotic pathogens. However, the search in Saskatchewan has revealed a relatively narrow range of pathogens on scentless chamomile, with only two fungal species that cause significant damage to the weed when applied by foliar inoculation. It is desirable to evaluate a more diverse group of microorganisms prior to a decision of further investment for development.

In the literature, a wide group of pathogens have been found on scentless chamomile or closely related species in Europe. Scientists at CABI Bioscience Switzerland Centre in Delémont also frequently observed severe disease on scentless chamomile in their research garden in the past several years. The causal agents were identified in collaboration with German Plant Protection Office at Lörrach, Dr. L.E. Petrini at Tecino, Switzerland and CABI Bioscience Egham, UK. Based on the information acquired, other publications/reports, and fungal collections held at CABI Bioscience Egham, UK (formerly known as the International Institute of Mycology (IMI), a list of potential fungal pathogens in Europe was constructed (Appendix 1).

A total of 31 fungal species were found to be associated with *M. perforata* and closely related plant species *M. maritima, M. recutita* and *M. matricarioides*. Many of them are plurivorous pathogens, attacking several plant species. Some are obligate parasites such as rusts and powdery mildews that can only grow and multiply in living plant tissues. Several pathogens, including *Ascochyta matricariae, Colletotrichum gloeosporioides, Fusarium oxysporum, Ramularia matricariae*, and *Septoria matricariae*, can be very host specific, attacking a narrow range of plant species. Some host-specific *Colletotrichum* spp. and *Ascochyta caulina* have been developed or researched as microbial herbicides (TeBeest and Templeton 1985, Mortentsen 1988, Vurro et al. 2001). Scientists at CABI Bioscience Switzerland recorded a disease in their research garden that caused necrosis, distorted leaves and stems, and stunted young plants. This disease later developed further into the flowers, affecting seed production.

These symptoms were also observed each year on rosettes and mature plants in the Rhine Valley of Southern Germany. At one field site in the valley, a severe disease was spotted on a dense population of scentless chamomile in spring, and the majority of young plants were attacked and killed prior to flowering (Hinz, CABI Bioscience, personal observations).

The above information pointed to the likelihood that a diverse and potentially more virulent group of pathogens of scentless chamomile might be found in Europe. The objectives of this project were to survey and collect disease-causal organisms from the area of weed origin, and evaluate them for biocontrol of scentless chamomile in western Canada. The field survey and pathogen isolation was conducted through collaborations with scientists at CABI Bioscience based in Switzerland. The evaluation of obtained organisms was carried out mostly in a pathology containment facility at AAFC Saskatoon.

Materials and Methods

Field surveys were conducted in three target areas where disease had previously been spotted on scentless chamomile. Survey time was spread to cover different growth seasons and diseases on both winter and summer annuals. Isolated disease-causal organisms were shipped to AAFC Saskatoon for evaluation. A bioassay using detached leaf tissues of scentless chamomile was conducted initially to weed out saprophytes. Pathogenic isolates were applied as a foliar spray to whole plants of scentless chamomile to assess their weed-control efficacy. Finally, crop safety and key environmental requirements of leading candidates were studied to determine their potential as biocontrol agents.

Field surveys in Europe

The first-year field survey was conducted between August 2000 and August 2001, and a total of 14 locations were visited in three areas covering several countries in Europe including Germany, France, Austria, UK, and Switzerland (Figure 1). All locations were naturally infested with scentless chamomile except the one in Switzerland where the weed was maintained in the research garden of CABI Bioscience in Delémont. Most infestations were in fallow fields, but also along field edges and roadsides, or by gravel pits. Disease samples were taken on several occasions throughout the year at Delémont and UK sites during the first

year, whereas collections from other sites were conducted intensively during May of 2001 when weather conditions were conducive to plant diseases. In 2002, the survey began in late April for potential early-season diseases. Due to a limited budget, field collections were mostly around the Rhine Valley area. Field trips were carried out for 4 weeks in the spring with 11 sites visited. In the majority of cases, disease samples were taken from *M. perforata*. In the Rhine Valley, diseases with severe symptoms on some herbal chamomile (*M. recutita*) were also collected when the plants were intermixed with scentless chamomile.

Figure 1. Location of field sites in Europe visited during field surveys for pathogens on scentless chamomile between 2000 and 2001.

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Figure 1. Location of field sites in Europe visited during field surveys for pathogens on scentless chamomile between 2000 and 2001.

Individual plants, especially young tissues from the new growth, were examined for disease symptoms. Disease leaves and stems were kept either in plastic bags in a cooler temporarily or in a plant presser until processing. Plants with wilt or dieback were collected and both above- and underground portions were examined. Infected samples were incubated for isolation of causal pathogens. Small pieces (approximately 3 x 3 mm) were cut from the edge of diseased tissues, surface sterilized, and incubated on Potato Dextrose Agar (PDA) containing 100 ppm of streptomycin and penicillin. Generally four tissue pieces of the same symptom were placed in a petri plate sealed with Parafilm® and incubated at room temperature. After about one week, plates were checked and pure cultures were transferred onto PDA.

Detached-tissue bioassay - pathogenicity testing

An import permit was obtained from CFIA for shipping foreign plant pathogens to Canada. Assessment of these organisms was carried out in a Level-2 containment facility at the Saskatoon Research Center following established safety guidelines. Initially, a bioassay was developed for efficient verification of pathogenicity. Detached leaves of scentless chamomile were surface disinfected using 10% Javex, then placed on moist filter papers in petri dishes. Mycelium plugs (about 5x5 mm) of purified fungal cultures were placed in close contact with the chamomile tissues, and the dishes were wrapped with the Parafilm® and kept at the room temperature. The leaf tissues were examined for disease symptoms originating from the mycelium plugs one and two weeks after inoculation, respectively. Organisms causing distinctive disease symptoms were considered potentially pathogenic and retained for further evaluation on weed control at whole-plant level.

Screening for weed control potential

This study was aimed at identifying efficacious agents for biocontrol of scentless chamomile at whole plant level. All isolates resulting in disease symptoms in the bioassays were tested.

Production of plants: Transplants of scentless chamomile were produced by sowing mature seeds in soilless media in plastic seeding trays. At about the 2-leaf growth stage, the seedlings were transplanted individually into 3-inch pots containing peat-based soilless mix amended with 3.5 kg of 16-8-12 (N-P-K) and 700 g of 0-20-0 fertilizers in 3.8 ft^3 of the growth medium. Transplants were kept in the greenhouse for about 3 weeks until reaching the 5 to 7 leaf stage.

Inoculum of fungal pathogens: All identified fungal pathogens were cultured on several agar media for sporulation. The cultures were incubated at 25/20°C with 14-h supplement near-UV lighting for about 2 weeks or until a sufficient amount of spores were produced. To prepare for inoculation, fungal cultures were flooded with water containing 0.1% Tween 80 surfactant, and scraped with a bent glass rod to dislodge the spores. The concentration of spore suspensions was estimated with aid of a hemocytometer and adjusted to approximately 1 x 10^6 spores/ml.

Inoculation of scentless chamomile: A spore suspension of an individual fungal pathogen was applied to the foliage of scentless chamomile (5-7 leaves) until runoff using an airbrush sprayer. Four plants were treated with each fungal candidate as replicates, and a water control was included with an individual trial. Inoculated plants were placed immediately in a dew chamber set at 20 C for 24 hours to allow the infection, then kept in growth cabinets for up to two weeks for disease development.

Assessment of weed control: The effect of fungal treatment was assessed by examining disease severity one and two weeks after the inoculation. In addition, plant fresh weight was taken after the second disease rating. The disease severity was measured for each individual plant scentless chamomile using a 0 to 6 pre-transformed rating scale (Little and Hill 1978) that corresponded to the damage severity of 0, 0-7%, 7%-25%, 25%-50%, 50%-75%, 75%-93%, and 93-100% on scentless chamomile. For fresh weight, plants were cut at the soil line and weighed separately for each replicate. Organisms causing significant disease symptoms or dramatic reduction of plant fresh weight were preserved in – 80°C ultra-low freezer for further evaluation.

Dose effect of *Alternaria* sp. The isolate 01-11FBr, identified as *Alternaria* sp., produced large-sized spores and spraying at the concentration of 1 x 10^6 spores/ml often caused plugging. An experiment was conducted using fungal suspensions containing 5 x 10^4, 5 x 10^5, and 5 x 10^6 spores/ml to determine if lower doses could provide equivalent weed control. Plants were inoculated using an airbrush sprayer and immediately provided with 24-h dew after the treatment. Generally there was no plugging at the lowest concentration. Occasional plugging occurred with 5 x 10^6 spores/ml, but spraying lasted only a few seconds with the highest concentration and the sprayer had to be cleaned several times to complete one application. The experiment was a completely randomized design with 4 replicates for each treatment.

Identification of fungal organisms

Efficacious fungal isolates were initially identified to fungal genera in the lab based on their cultural characteristics and spore morphology. For confirmation of the identification and species designation, multiple strains of indigenous and foreign *Colletotricum* sp. were also sent to the National Fungal Identification Services at AAFC, Centraalbureau Voor Schimmelcultures (CBS) at the Institute of the Netherlands Academy of Arts and Sciences, and to Fungal Identification Services at CABI Biosciences in UK.

Host-range studies

In the first year, six isolates that caused more than 50% of fresh-weight reduction on scentless chamomile were tested for host-range to determine their safety to crops and native plants in Saskatchewan. The pathogens were evaluated initially on 4 common crop species, i.e. wheat, canola (*Brassica napus*), lentil and flax. In previous studies, both lentil and flax were more sensitive to several fungal pathogens from the chamomile. Isolates exhibiting no negative effect on these crops were subjected to expanded host-range testing involving 15 plant species belonging to the families *Asteraceae* (3), *Brassicaceae* (3), *Fabaceae* (4), *Poaceae* (3), and *Linaceae* (1) following the centrifugal-phylogenetic scheme described by Wapshere (1974). In 2002, due to a limited number of fungal candidates selected, the initial step was omitted and all three isolates were tested on 13 plant species.

To produce test plants, seeds of scentless chamomile, ox-eye daisy and pineapple weed are scattered over packed and pre-watered Pro-Mix amended with similar fertilizers described above. Prior to the emergence of the 3rd leaf, seedlings were transplanted to 3-inch pots (1 plant/pot for scentless chamomile) or 4" pots (5 plants/pot for pineapple weed and ox-eye daisy) containing the same soilless mix. Other host-range plants were seeded directly in 5" pots containing the Pro-Mix with 5 to 10 plants per pot. The plants were maintained in the greenhouse until inoculation.

Spore suspensions of fungal pathogens were applied to the foliage of all test plants till runoff using an airbrush sprayer. Conducive conditions, including high inoculum doses (5×10^5 to 1×10^6 spores/ml) and a 48-h dew period after inoculation, were provided to favor the infection. Treated plants were kept in the growth cabinets in the containment for 2 weeks to allow for expression of disease symptoms. Depending on species, plants were inoculated generally at the 4 to 6-leaf stage. Three replicated pots were used for each plant species in a trial.

Additional biological assessment

Minimal requirement of leaf wetness for infection: In two years of evaluation, a total of 6 isolates passed host-range test and exhibited a similar level of weed control. These isolates all belonged to *Colletotrichum* sp. This experiment was designed to differentiate these isolates based on leaf-wetness requirements for infection, and a reduced requirement would certainly be a desirable trait for biocontrol agents under the prairie climate. The study was conducted at 20°C, a relatively favorable ambient temperature for germination of these fungal pathogens. Scentless chamomile at the 7-leaf growth stage was inoculated with these fungi at 1×10^6 spores/ml. Immediately after inoculation, plants were placed in a dew chamber for 0, 12, 16, 20 or 24 h to provide variable leaf wetness durations (dew periods). Plants taken out of the dew chamber were placed in growth cabinets in the containment and examined for disease a week after inoculation. Plant fresh weight was also taken at the end of experiment. For each isolate, four replicated plants were used for each dew period tested.

Comparison with indigenous strains: A total of six foreign isolates of *Colletotrichum* sp. were compared with indigenous strains for the dew requirement and weed control. For the comparison of dew requirement, experimental procedures similar to those described in the above experiments were used. Statistical analyses were performed on individual fungal isolates with a focus on the initiation of disease symptoms and the peak of disease damage in relation to the duration of leaf wetness. For weed control, the foreign isolates were evaluated side by side with indigenous strains on scentless chamomile at two growth stages, i.e. the 6- to 7-leaf stage and 11- to 13-leaf stage, respectively. Aqueous spore suspensions of the fungal isolates, amended with 0.1% Tween 80® surfactant and adjusted to approximately 1×10^6 spores/ml, were applied to scentless chamomile plants using an airbrush sprayer following the inoculation method described above. Treated plants were provided with 24-h dew at 20°C in a dew chamber, then kept in a growth cabinet with the same condition as described above. Disease severity was examined one and two weeks after inoculation, and plant fresh weight was taken after two weeks. The experiment was a factorial design with 4 replicated plants for each treatment. Statistical analyses were carried out to examine the effect of fungal isolates and growth stage of the weed, as well as the potential interaction between the two factors.

Experimental designs and data analyses

Randomization was applied throughout the study and a computer software STATISTICA (StatSoft, Tulsa, OK) was used to perform statistical analyses for all experiments. Data were initially examined for the independence of variance from the means and arcsine or log transformation might be applied when the independence was not satisfied. Data from single-factored experiments were initially subjected to analysis of variance (ANOVA) at $P < 0.05$. When the ANOVA revealed significant differences, means of individual treatments were then separated using the least significant difference (LSD, $P = 0.05$). For experiments involving a factorial design, initial ANOVA tables were used to determine the significance of the main factors and their interactions. Significant interactions were noted in results and trends were discussed. After initial screening, experiments were generally repeated once unless noted otherwise. Data from repeated trials were tested for the homogeneity of variances before pooling or analyzed separately. Unless otherwise noted, data from repeated trials were pooled prior to analyses.

Results and Observations

2000-2001 results

Field surveys: Three major infestation areas in western-central Europe, including the center of weed origin around eastern Austria and Hungary, were targeted in first-year surveys. All the areas had abundant natural infestation of scentless chamomile. Several types of diseases were observed on multiple sites of each area, including chlorosis, leaf spots, stem lesions, and plant wilt or diebacks. In comparison, more severe diseases were observed in the Rhine Valley and, at the CABI research garden where scentless chamomile had been maintained continuously for several years. In eastern Austria where spectacular disease symptoms had been spotted before, diseases were hardly spotted during a survey in late May of 2001, a circumstance possibly attributable to a relative dry spring in the area. In southern England, diseases were common, especially for leaf spots. The disease severity, however, was quite light. A total of 138 fungal isolates were obtained from disease samples. Based on preliminary identification, they belonged to at least 13 fungal genera. Most isolates were from *M. perforata* while several were from *M. recutita*. Some of the fungal genera had not been previously reported in association with scentless chamomile.

Pathogenicity bioassay: Of the 138 fungal isolates screened, approximately 50% exhibited a level of pathogenicity on detached leaves of scentless chamomile (Table 1), with necrosis as the most common symptom observed (Photo 1). In many cases, symptoms were not visible until 2 weeks after incubation. Several isolates caused damage rapidly and that appeared to be due to phytotoxins from the fungal isolates. Most of the isolates examined were from the Rhine Valley and CABI research garden as a result of high disease incidence and severity at these locations. Fungal isolates that caused no disease after two weeks were autoclaved and discarded. In total, 68 isolates were considered pathogenic based on the bioassay results (Table 1).

Table 1. Summary of bioassay results on the pathogenicity of fungal isolates collected in Europe during 2000 and 2001.

Country Area surveyed (coordinates)	**No. of sites**	**Isolated from** (plant species)	**No. of fungal isolates obtained**	**No. of pathogenic isolates identified**
Switzerland Delémont (47°21'N, 7°22'E)	1	*M. perforata*, *M. recutita*,	83	32
Germany/France Rhine Valley (47°48'N, 7°36'E)	7	*M. perforata*, *M. recutita*,	48	33
Austria Eisenstadt (47°51'N, 16°31'E)	3	*M. perforate*	3	1
United Kingdom Silwood Park (51°26' N, 0°46' W)	3	*M. perforata* *M. recutita*, *M. matricarioides*	4	2

Weed control on whole plants: When applied as aqueous spore suspensions, pathogenic isolates with positive results in the bioassay exhibited quite variable weed control on whole plants of scentless chamomile, ranging from highly effective (Photo 2) to completely ineffective. Most of these pathogens caused little disease on whole plants (Table 2), while six isolates resulted in significant weed control, reducing the fresh weight of scentless chamomile by 44.7% to 95.2% in comparison with non-treated controls (Figure 2). Only 16% of the tested isolates resulted in statistically significant damage to scentless chamomile. Symptoms were mostly leaf spots, with slightly sunken lesions visible on leaflets and petioles 4 days after inoculation. The lesions expanded slowly, reaching 1 to 3 mm in size one week after inoculation. These lesions would coalesce when at high densities, killing diseased leaflets, petioles, and occasionally whole plants. Preliminary identification suggested that these isolates belong to fungal genera *Alternaria* and *Colletotrichum*, respectively. These two pathogens are cosmopolitan in distribution. Pathogens with low levels of weed control on whole plants were discarded.

Table 2. Control of scentless chamomile with by pathogenic fungal isolates identified through the bioassay process.

Total No. of isolates tested	Average disease severity [a]					
	None (0)	Trace (<5%)	Slight (5-25%)	Moderate (25-50%)	Severe (50-75%)	Extreme (>75%)
68	23 [b]	27	7	5	6	0

a. On whole plants at 5 – 7 leaf growth stages

b. Number of fungal isolates included.

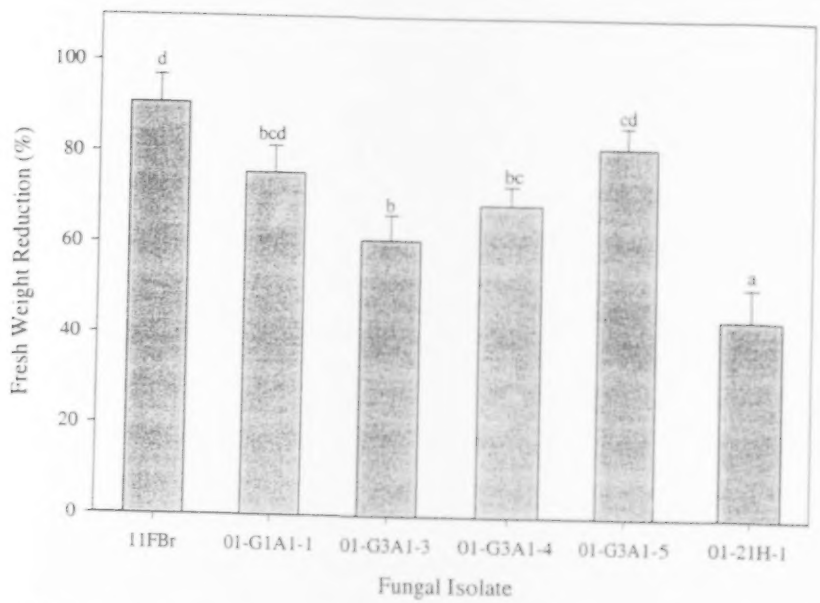

Figure 2. Effect of efficacious fungal pathogens on whole plants of scentless chamomile.

Dose effect of *Alternaria* sp. The spore dose of the isolate 01-11FBr had significant effect on weed control. At the concentration of 5×10^4 spores/ml, although the plugging did not occur, the weed control was noticeably poorer when compared with higher concentrations (Photo 3).

In comparison to untreated controls, the fungus from low to high spore concentrations reduced plant fresh weight by an average of 79% to 95% two weeks after application. This requirement of high inoculum dose is particularly a challenge for large-spore organisms like *Alternaria* sp. due to frequent plugging of spray equipment.

Host range determination: In preliminary tests on 4 common crop species, i.e. wheat, flax, canola, and lentil, five of the six most efficacious fungal isolates were completely non-pathogenic with no negative effect observed on any of the crop species. These five isolates appeared to belong to the fungal genus *Colletotrichum* based on spore morphology and the formation of acervuli. *Alternaria* sp. (isolate 01-11FBr), on the other hand, caused severe damage to canola, and moderate injuries on lentil and flax, therefore was not advanced for further evaluations.

On expanded host-range tests involving 15 plant species, these five *Colletotrichum* isolates produced almost identical results, causing disease only on scentless chamomile and German chamomile (*M. recutita*). In comparison, they were more virulent on scentless chamomile with substantially higher disease severity. All other plant species appeared to be immune to these fungal isolates with no visible disease or significant fresh-weight reduction recorded during two weeks of monitoring. Even on canola and lentil, which were susceptible to several fungal isolates from scentless chamomile, no abnormality was observed (Photo 4). Two related weedy species, pineapple weed and ox-eye daisy, showed no disease symptoms either. In selecting test-plant candidates for this study, both plant phylogeny and the importance of non-target Saskatchewan crops were taken into consideration. The crop species included common cereals, oilseeds, pulses and forages (Table 3), and the results strongly suggested that these isolates are host specific and safe to the crops grown in the province. Infection of German chamomile can be of concern to some herb growers. However, the production of German chamomile is limited to a small acreage around Prince Albert area (<5 acres), and future acreage will likely remain small based on the market trend (Detillieux, herb chamomile seed dealer, personal communication). It is believed that field applications of these fungal organisms can easily avoid these pocket production areas.

Table 3. Reaction of selected crop and weedy species to foliar inoculation by fungal isolate 01-G1A1-1, 01-G3A1-3, 01-G3A1-4, 01-G3A1-5, and 01-21H-1 obtained from scentless chamomile in Europe during 2000 and 2001.

Plant family **Common name**	**Plant species**	**Cultivar**	**Plant reaction**
Asteraceae			
Scentless chamomile	*Matricaria perforate*	N/A	Very susceptible
German chamomile	*M. recutita*	N/A	Susceptible
Pineapple weed	*M. matricarioides*	N/A	Immune
Ox-eye daisy	*Chrysanthemum leucanthemum*	N/A	Immune
Brassicaceae			
Canola (Argentinean)	*Brassica napus*	Westar	Immune
Canola (Polish)	*B. rapa*	AC Parkland	Immune
Mustard	*B. juncea*	Cutlass	Immune
Fabeaceae			
Lentil	*Lens culinaris*	Eston	Immune
Field pea	*Pisum sativum*	Alfetta	Immune
Chick pea	*Cicer arietinum*	Sanford	Immune
Alfalfa	*Medicago sativa*	Beaver	Immune
Linacea			
Flax	*Linum usitatissimum*	Vimy	Immune
Poaceae			
Wheat (prairie spring)	*Triticum aestivum*	AC Crystal	Immune
Barley	*Hordeum vulgare*	Harrington	Immune
Oats	*Avena sativa*	Calibre	Immune

Minimal requirement of leaf wetness

At 20°C, three *Colletotrichum* isolates behaved slightly differently in terms of their requirement for leaf wetness. In general, infection did not occur when the wetness duration was shorter than 8-h (Figure 3). Between 8 and 12-h dew, only sparse infection occurred with very low disease severity. After 12 hours, the infection rose quickly with the leaf-wetness duration, especially on plants inoculated with the isolate 01-G1A1-1. A significantly higher level of disease occurred with this isolate at 16-h dew when compared with the other two isolates. Highest infection appeared to occur between 20 and 24-h leaf wetness, especially with the isolates 01-G1A1-1 and 01-G1A1-3. The isolate 01-21H-1 was the least virulent strain, resulting in only light damage with even 24-h leaf wetness. Likely, the required leaf wetness would be longer under less favorable temperatures. The higher disease caused by 01-G1A1-1 at 16-h dew suggests that this isolate is able to establish the infection more rapidly on scentless chamomile.

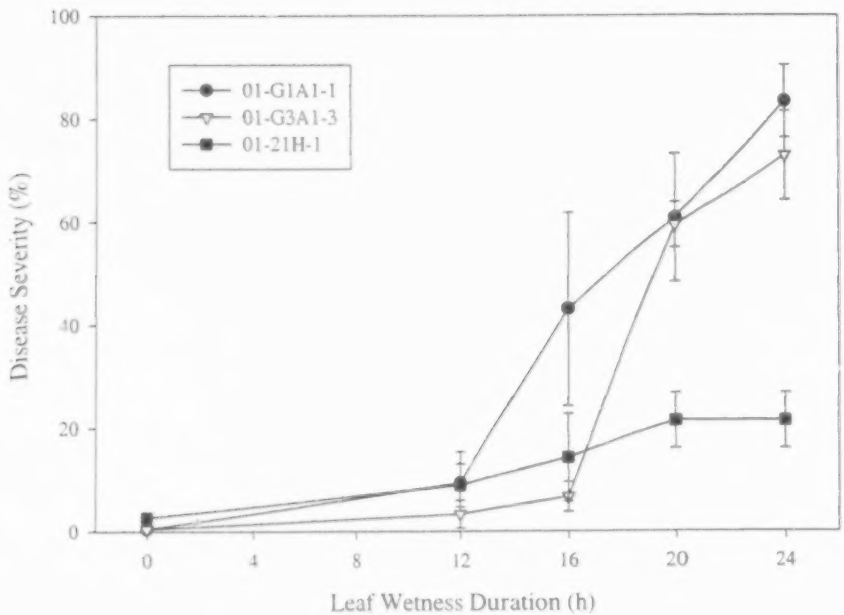

Figure 3. Effect of leaf wetness duration on disease severity of scentless chamomile caused by three isolates of *Colletotrichum* sp. found in European surveys in 2001.

Comparison of foreign isolates with indigenous strains: In comparison with two leading indigenous stains, the foreign isolates of *Colletotrichum* sp. were morphologically similar. There were almost identical biological traits among these strains in terms of the host range and dew requirement for infection. All strains required a 20- to 24-h dew period to cause maximal infection on scentless chamomile, even under relatively favorable temperatures. Two foreign isolates, 01-G1A1-1 and 01-G3A1-3, demonstrated similar levels of weed control as did the indigenous strains on scentless chamomile at 6- and 13-leaf growth stages, respectively (Figure 4, Photo 5). The isolate 01-21H-1 was less effective, especially on older plants of scentless chamomile, with 50% less weed control when compared with the other two strains of *Colletotrichum* sp.

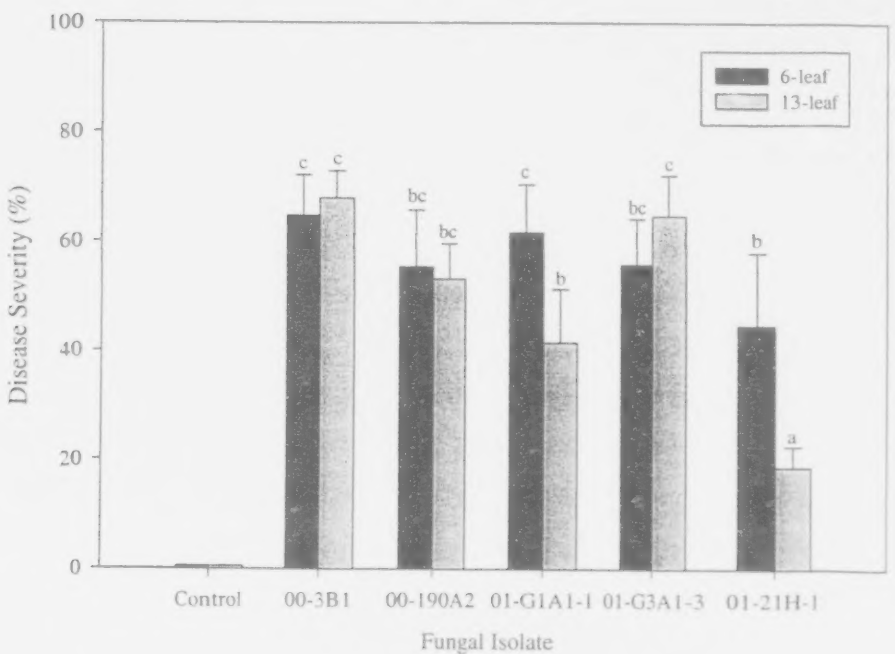

Figure 4. Disease severity caused by three foreign isolates of *Colletotrichum* sp. (01-G1A1-1, 01-G3A1-3 and 01-21H-1) in comparison to that caused by two indigenous strains (00-3B1, 00-190A) on scentless chamomile of 6-leaf and 13-leaf growth stages.

2002-2003 results

Field surveys and bioassays: Field surveys in 2002 started in early April to collect disease samples from early-season infection. The focus was on *Colletotrichum* diseases due to the host specificity of the pathogen and relatively higher efficacy of weed control in previous evaluations. Multiple sites were visited in the Rhine Valley area with 71 fungal isolates obtained. The bioassay on detached leaves revealed 14 fungal isolates as potential pathogens on scentless chamomile, and these isolates were shipped to Saskatoon for further testing on whole plants in the pathology containment.

Weed control on whole plants

1. **Preliminary assessment**

Selected pathogenic isolates exhibited variable weed control when applied as foliar sprays. In general, isolates with relatively higher levels of weed control belonged to *Colletotrichum* sp. (Table 4). *Fusarium* sp., *Phoma* sp. and *Septoria* sp. caused only light infection, therefore were not tested further for foliar applications. It needs to be noted that soil applications were not tried in this study and some of these organisms may be more virulent as soil pathogens. In comparison to an indigenous strain (00-3B1), all *Colletotrichum* isolates were at least as effective based on the fresh-weight reduction.

2. **Biocontrol of scentless chamomile at different growth stages**

Weed-control potential of 5 most efficacious *Colletotrichum* isolates were tested on scentless chamomile of at 7-leaf and 11-leaf stages. In general these isolates were equally effective on plants of the different growth stages (Figure 5), causing a similar level of disease and fresh weight reduction. The isolate 02-G9D1 was slightly less efficacious while the isolates 02-G11H1, 02-G1C1, and 02-G9H1 were slightly more effective in reducing the fresh weight on older plants. In comparison to the untreated controls, the foreign isolates reduced the fresh weight of scentless chamomile by 55% to 73%.

Table 4. Efficacy of selected fungal isolates on whole plant of scentless chamomile

Isolate	Fungal genus	Concentration (10^6spores/ml)	Disease severity (%)	Fresh weight (g)
Control (blank)	N/A	0.0	0.0	1.3
00-3B1 [a]	*Colletotrichum*	1.5	50.0	0.4
02-E1E1	*Phoma*	0.7	9.8	1.0
02-E1E2	*Phoma*	1.7	1.2	1.1
02-G11D1	*Fusarium*	1.0	15.1	1.4
02-G11H1	*Colletotrichum*	3.0	78.6	0.3
02-G1A2	*Phoma*	1.5	2.6	1.6
02-G1C1	*Colletotrichum*	0.9	44.6	0.8
02-G8A1	*Fusarium*	3.0	29.9	1.1
02-G8A2	*Fusarium*	0.8	8.9	1.1
02-G8C1	*Phoma*	3.0	12.9	1.0
02-G9D1	*Colletotrichum*	2.7	79.5	0.2
02-G9E2	*Colletotrichum*	0.8	56.3	0.3
02-G9E4	*Fusarium*	2.7	9.85	1.5
02-G9H1	*Colletotrichum*	2.9	88.0	0.2
02-G9I2	*Septoria*	0.2	21.4	1.0

a. An indigenous strain of *Colletotrichum* sp. isolated from scentless chamomile.

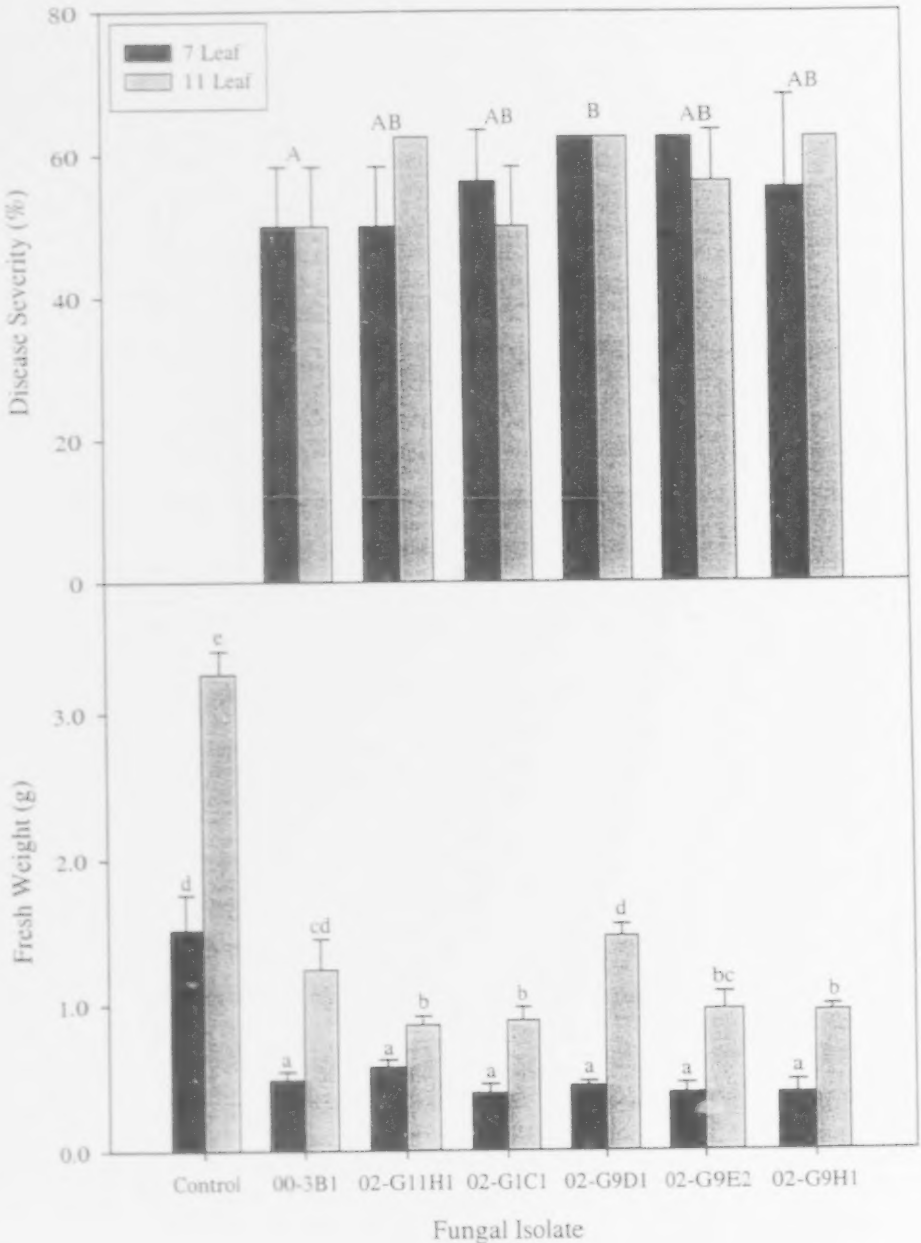

Figure 5. Disease severity (upper graph) and fresh weight reduction (lower graph) by five foreign isolates of *Colletotrichum* sp. in comparison with the indigenous strain (00-3B1) on scentless chamomile at 7- and 11-leaf growth stages.

Identification of *Colletotrichum* sp.

By comparing isolates of different origins, both Dr. P.F. Cannon at CABI Bioscience and Dr. J. Bissett at AAFC Fungal Identification Services believed the isolates from Europe were the same as those found in Saskatchewan (Appendix III). The designation to species, however, was less conclusive when based only on the spore morphology. Initially the indigenous isolate 00-3B1 was examined as a model strain by taxonomists at CBS. They suggested this strain is intermediate between *C. capsici* (Syd.) Butl. & Bisby and *C. circinans* (Berk.) Vogl, but further emphasized that morphological species identification on the falcate-spored capsici group of *Colletotrichum* spp. was unreliable. Dr. Cannon first thought the strain 00-3B1 was *C. truncatum*. After examining multiple isolates from Europe and Saskatchewan, he was less confident about this species designation and explained that the conidia were similar to those of *C. truncatum*, but the *Matricaria* strains tended to have slightly straighter conidia with slightly more blunt ends.

It is likely that these *Colletotrichum* strains belong to a species fairly similar to *C. truncatum*. Results from the host-range study below seem to suggest that they are different from typical *C. truncatum* that causes severe infection on lentil. Biometric studies including DNA sequencing may be required to confirm species designation accurately. There are several sequences of *C. truncatum* and other closely-related species available in GENBANK, although there may not be independent confirmation of their correct identity.

Host-range studies: Among the 13 plant species tested, the three *Colletotrichum* isolates exhibited a fairly similar host range and caused infection only on scentless chamomile and German chamomile (Table 5) but with much lighter disease on the latter host. This lower virulence on German chamomile was similar to other *Colletotrichum* isolates evaluated in the previous year. Other plant species, including all the pulse crops tested, were immune to these fungal isolates with no disease or fresh weight reduction recorded two weeks after treatment.

Minimum dew requirement: No infection was observed with any of the isolates when the dew period was shorter than 8 h post inoculation (Figure 6). Light infection occurred on

plants treated with the isolate G9H1 at 12-h dew, while other isolates required up to16-h dew to cause visible disease symptoms. For isolates G9H1 and G11H1, further increase of the dew duration from 16 h to 24 h enhanced the disease severity and the weed control reached the maximum level at 24-h dew. For isolate G1C1, the disease severity differed only marginally between 16 and 24-h dew.

Table 5. Reaction of selected plant species to *Colletotrichum* sp. isolated from scentless chamomile in European in 2002.

Plant family *Plant species*	**Common name**	**Cultivar**	**02-G1C1**	**02-G9H1**	**02-G11H1**
Asteraceae *Matricaria perforata*	Scentless chamomile	N/A	VS [a]	VS	VS
M. recutita	German chamomile (Herb)	N/A	S	S	S
Chrysanthemum leucanthemum	Ox-eye daisy	N/A	I	I	I
Brassicaceae *Brassica napus*	Argentinean canola	Westar	I	I	I
B. juncea	Mustard	Cutlass	I	I	I
Fabeaceae *Lens culinaris*	Lentil	Eston	I	I	I
Pisum sativum	Field pea	Alfetta	I	I	I
Cicer arietinum	Chick pea	Sanford	I	I	I
Medicago sativa	Alfalfa	Beaver	I	I	I
Linacea *Linum usitatissimum*	Flax	Vimy	I	I	I
Poaceae *Triticum aestivum*	Wheat (spring)	AC Crystal	I	I	I
Hordeum vulgare	Barley	Harrington	I	I	I
Avena sativa	Oats	Calibre			

[a] Plant reaction to foliar inoculation: I – immune, S – susceptible, VS – very susceptible.

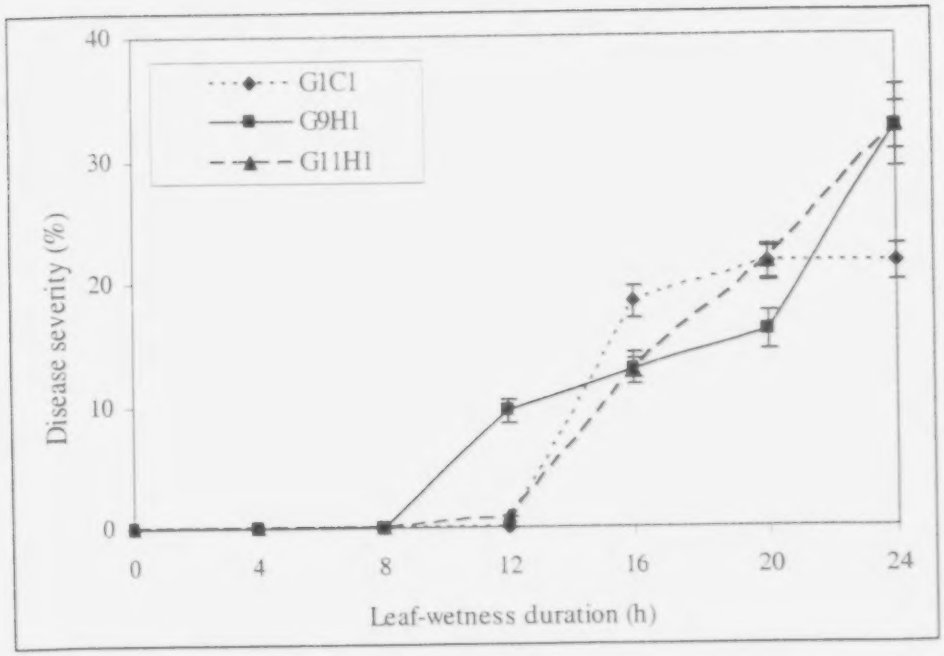

Figure 6. Effect of leaf wetness duration on disease severity of scentless chamomile caused by three isolates of *Colletotrichum* sp. imported from Europe in 2002.

Conclusions

The survey and evaluation of plant pathogens from the area of weed origin for biocontrol of scentless chamomile was completed through the collaboration between AAFC Saskatoon and CABI Bioscience Switzerland. A group of *Colletotrichum* sp. isolates, with moderate weed-control efficacy and strong host specificity, stood out as potential biocontrol candidates. These foreign isolates, with the same morphological characteristics as the *Colletotrichum* strains found in Saskatchewan, likely belong to a species closely related to *C. truncatum*. Final species designation may require molecular proof in comparison to known *Colletotrichum* species.

In comparison to indigenous stains, the foreign *Colletotrichum* isolates were equally efficacious in controlling scentless chamomile. Although this was reassuring, we had hoped to discover a more diverse group of pathogens with higher virulence in the area of weed

origin. In addition, like their indigenous counterparts, these foreign isolates also required a extended dew period (up to 20 hours) to cause maximum infection on scentless chamomile, even under favorable temperatures. This stringent dew requirement can be a challenge for biocontrol on the prairies where rainfall is infrequent during the growing season and the relative humidity often low. Dew periods occur during cooler nights but continuous leaf wetness of 10 h or more is uncommon. It is clear that formulations will have to be developed for biocontrol applications under field conditions. It also need to be noted that soil application method was not tried in this study because it would required at least 50% more resources. Some of the fungi obtained, including *Fusarium* spp. and *Phoma* spp., can be more virulent pathogens when attacking plants from roots. This may be one of the aspects worth further investigation.

Based on the results of this study, also in consideration of additional regulatory requirements associated with foreign pathogens, we recommend that the development of biocontrol should be focused on indigenous *Colletotrichum* strains at this time. A project is already underway to explore potential uses of *Colletotrichum* sp. with synergistic herbicides for improved weed control. The potential of several fungal isolates as soil-applied biocontrol candidates should be evaluated since severe vascular-disease symptoms have been observed in Europe.

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Publications and presentations

1. Peng, G. 2001. Biological control of scentless chamomile using microbes. Saskatchewan Alfalfa Seed Producers Association Newsletter Vol. 14 (3):11-12.
2. Bailey K.L. Presentation on "Potential for biological control of scentless chamomile using plant pathogens" at Saskatchewan Scentless Chamomile Working Group meeting, April 2001.
3. Peng, G. Presentation on "Progress in research of biocontrol of scentless chamomile using exotic and indigenous plant pathogens" at Saskatchewan Scentless Chamomile Working Group meeting, October 2001.
4. Peng, G., K.L. Bailey, and H. L. Hinz. 2002. Evaluation of plant pathogens for biocontrol of scentless chamomile in western Canada. Poster at 2002 annual meeting of Canadian Phytopathological Soc., Waterton. AB
5. Peng, G. 2003. Control of scentless chamomile using a host-specific fungus and fungus/herbicide synergy. Saskatchewan Alfalfa Seed Producers Association Newsletter Vol. 16 (1):5-8.

Personnel (salaries/wages were paid by the ADF fund)

Faye Dokken, Casual technician at AAFC Saskatoon. Paid by the project for about 3 weeks with a total wage cost of $1,348.77.
Gavin Graham, Research Affiliate at AAFC Saskatoon. Paid by a salary for 14 months with a total cost of $17,351.26.
Wendy Guo, Casual Research Assistant at AAFC Saskatoon. $1,522.06 for about 4 weeks.
Christy Jackson, Casual Research Assistant at AAFC Saskatoon. $3,800.16 for 9 weeks.
Michael Cripps, Jürg Hiltbrunner, and Jean-Philippe Marelli. Summer students at CABI Bioscience Switzerland Center. Partially sported by the project ($15.059.60 as fees).

Equipment

No equipment or supply over $500 was purchased using the this project funding.

Project Developed Materials:

Several isolates of *Colletotrichum* sp. have been identified as candidates for biocontrol of scentless chamomile and preserved at AAFC Saskatoon. Their potential, especially in

relation to the synergy with herbicides will be evaluated further once the taxonomy is confirmed.

Project Photos

Five illustration photos are included in Appendix 2 of this report.

Acknowledgement

This project was financed with funding from several organizations. Monetary contributions by ADF of Saskatchewan Department of Agriculture and Food, Matching Investment Incentive of AAFC, and Philom Bios Inc. to this project are gratefully acknowledged.

Expense Statement

A financial statement is included in Appendix 4. The funding was fully utilized and will be in zero balance once the last installment is paid upon receiving this report by the ADF. The salary portion was a little higher than what was budgeted mainly because additional technical assistance was required to process a large number of samples at AAFC Saskatoon during the 2001 survey. Slightly higher expenses in the 'Other' category was due to additional costs incurred in 2002 field survey. However, the overall budget was balanced by reducing expenditures in fees and materials/supplies.

Appendix 1. Fungal pathogens recorded in association with *Matricaria perforata* and related weed species in Europe.

Name	Host plants recorded/Host range	Symptoms	Sources
Albugo tragopogonis Pers. ex S.F. Gray	Generally on: *Tragopogon* , but also *Chamomilla suaveolens, Antennaria, Artemisia, Centaurea, Helianthus, Senecio,* etc.	"White rust", causing white blisters	Farr *et al.* 1989, Ellis and Ellis 1987, Brandenburger 1985, Horst 1979, index of plant diseases 1960
Alternaria alternata (Fr.) Kreissler	Plurivorous, Opportunistic	Necrotic spots on leaves and stems	Isolated from *M. perforata* in 1994 and '97 (diseased seedlings)
Ascochyta matricariae Grove	The species can be host specific	Leaf necrosis; can be quite damaging	Isolated from *M. perforata* in 1994
Botrytis cinerea Fr.	Plurivorous, common grey mould	Leaf spot, fruit rot, petal specks	Isolated from *M. perforata* in 1993; Kay 1994
Cladosporium sp.	Ubiquitous and mostly saprophytic	Associated with leaf spots, necrosis, and lesions in plant tissues	Isolated from seedlings of *M. perforata* in 1997
Colletotrichum gloeosporioides (Penz.) Penz & Sacc.	Host-specific forms exist	Usually anthracnose with black and slightly sunken lesions.	Isolated from *M. perforata* in 1996
Entyloma matricariae Rostr. = *E. matricariae* Trail = *E. trailii* Mass.	According to Buhr (1964) only on *M. perforata.*	White smut, typically leaf chlorosis and necrosis	Ellis and Ellis 1987, Hegi 1987,Brandenburger 1985, Buhr 1964, Lindeberg 1959; IMI ref. no. 329906
Erysiphe cichoracearum	plurivorous; among many others on: *Chamomilla* spp. and *Achillea* spp.	Powdery mildew	Farr *et al.* 1989, Ellis and Ellis 1987, Brandenburger 1985, Horst 1979, Index of plant diseases 1960

..... Continues

Erysiphe polyphaga Hammarlund	plurivorous [*Oidium* is the anamorphic state]	Powdery mildew	Blumer 1967, Brandenburger 1985
Fusarium oxysporum Schlecht.	Can be host-specific, forma specialis known	Vesicular infections, distorted leaves/stems	Isolated from *M. perforata* in 1994
Gliocladium roseum Bainier	Mycoparasite	Not details	Isolated from *M. perforata* in 1994
Helotium sp.	*Matricaria maritima*	No details	IMI record
Microdochium sp.	Cereals	No details	Isolated from *M. perforata* in 1999
Monographella nivalis (Schaffn. E. Müll.)	Mainly known from cereal crops	No details	Isolated from *M. perforata* in 1996
Mycosphaerella sp.	*Matricaria maritima*	Leaf spot	IMI record
Oidium sp.	Plurivorous	Powdery mildew	Brandenburger 1985, Horst 1979, Blumer 1967
Olpidium brassicae (Woronin) P.A. Dang.	Plurivorous; besides many others, *Chamomilla suaveolens*	Disease on seedlings	Ginns 1986
Peronospora leptosperma De Bary	*Matricaria* spp., *Chamomilla* spp., *Anthemis* spp., *Tanacetum* spp.	Downy mildew	Ellis and Ellis 1987, Hegi 1987, Brandenburger 1985, Buhr 1964, Docters van Leeuwen 1957, Frank 1880; IMI ref. No. 353388
Peronospora radii De Bary = *P. danica* Gäum.	*Matricaria* spp., *Chamomilla* spp., *Achillea* spp., *Anthemis* spp., *Chrysanthemum leucanthemum, C. segetum*	Downy mildew; deformed ray and disc florets	Hegi 1987, Brandenburger 1985, Buhr 1964, Butler & Jones 1949, Frank 1880, IMI record
Phoma sp.	Plurivorous	Necrotic leaf spot	Isolated from *M. perforata* in 1993 and 2001; IMI record
Phomopsis albicans Roberge ex Desm	*M. perforata, Hypochaeris*	Necrotic stem lesions	Grove 1935, Ellis & Ellis 1987, isolated from *M. perforata* in 2001

Pleospora herbarum (Pers.)	*Matricaria maritime, Chamomilla recutita*	Necrotic stem lesions	Hegi 1987, IMI record, isolated in 2001 as *Stemphylium* sp.
Protomyces matricariae Syd.	*M. perforata*	Pale green, producing calluses on leaves	Buhr 1964, Brandenburger 1985
Puccinia matricariae Sydow = *P. tanaceti* ?	*Chamomilla recutita*	Rust	Brandenburger 1985, Gäumann 1959, IMI record
Ramularia matricariae Antokol ex W. Vassilijevsky & Karak = *Didymaria matricariae*	*Matricaria* spp.	White leaf spots/lesions;	Isolated from *M. perforata* in 1999, 2000; IMI ref. No's 7550 and 7551
Septoria matricariae Sydow	*Matricaria perforata, Chamomilla* spp., *Anthemis cotula*	Leaf spot	Ellis & Ellis 1987, Brandenburger 1985; IMI ref. No. 353746
Sphaerotheca erigerontis-canadensis	*Chamomilla suaveolens, Erigeron canadensis,*	Powdery mildew;	Ellis and Ellis 1987
Sphaerotheca fuliginea	*Chamomilla recutita, C. suaveolens, Veronica chamaedrys, Caltha*	Powdery mildew	Farr *et al.* 1989, Ellis and Ellis 1987, Ginns 1986, Brandenburger 1985; IMI record
Sphaerotheca humuli (D.C.) Burr. *var. fuliginea* (Schlecht.) Salm.	*Chamomilla suaveolens*	Powdery mildew	Horst 1979, Index of plant diseases 1960
Sphaerotheca macularis	Plurivorous; besides others on: *Chamomilla suaveolens and Anthemis cotula*	Powdry mildew;	Farr *et al.* 1989, Ellis and Ellis 1987, Horst 1979
Sphaerotheca xanthii	*Chamomilla suaveolens*	Powdery mildew;	IMI record

[a] CABI Bioscience, 2001: Database of fungal names (Funindex): http://194.131.255.3/cabipages/Names/Names.asp

Appendix 2. Photos

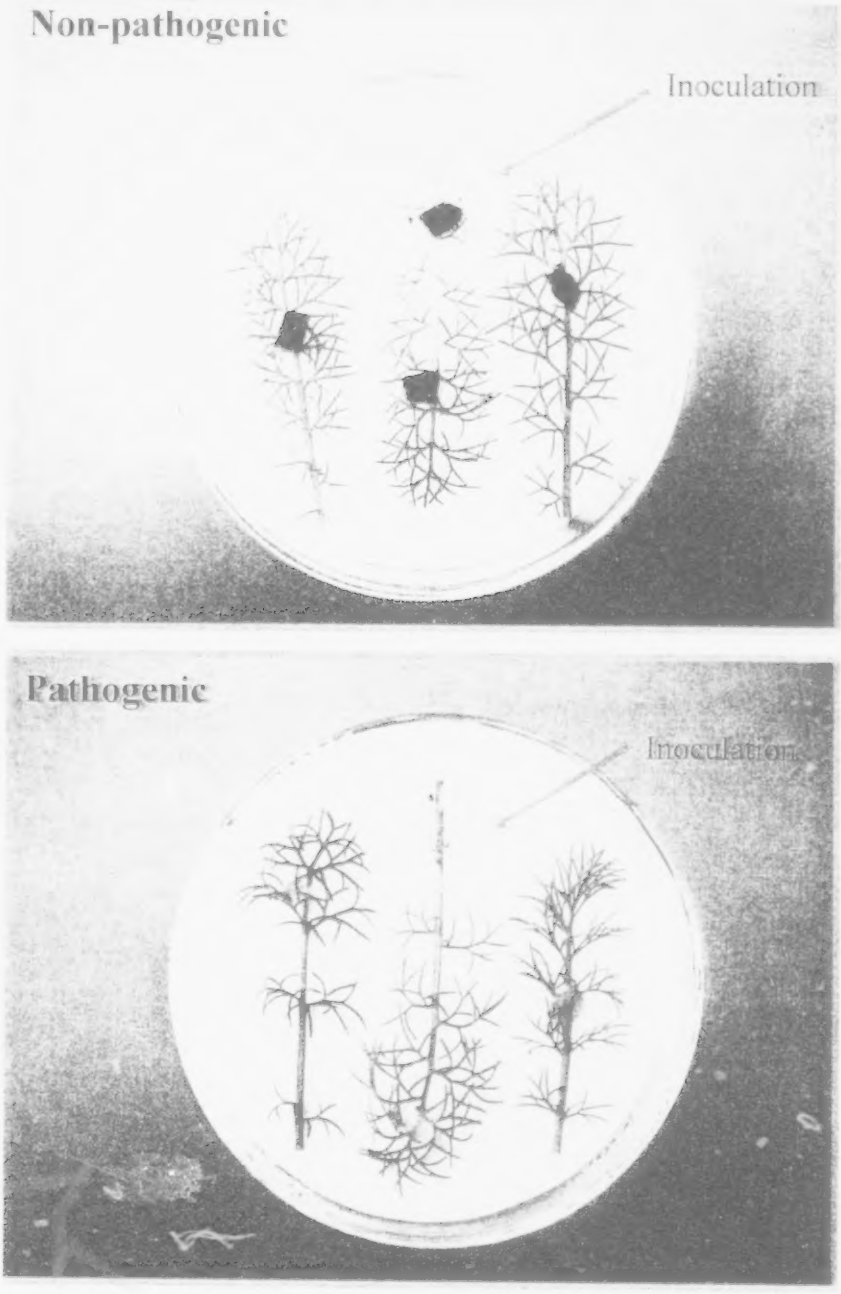


Photo 1. Bioassay for pathogenicity testing. The organisms in the top and bottom plates are considered non-pathogenic and pathogenic, respectively, according to the presence of disease symptoms originating from the inoculation.

Appendix 2. Photos

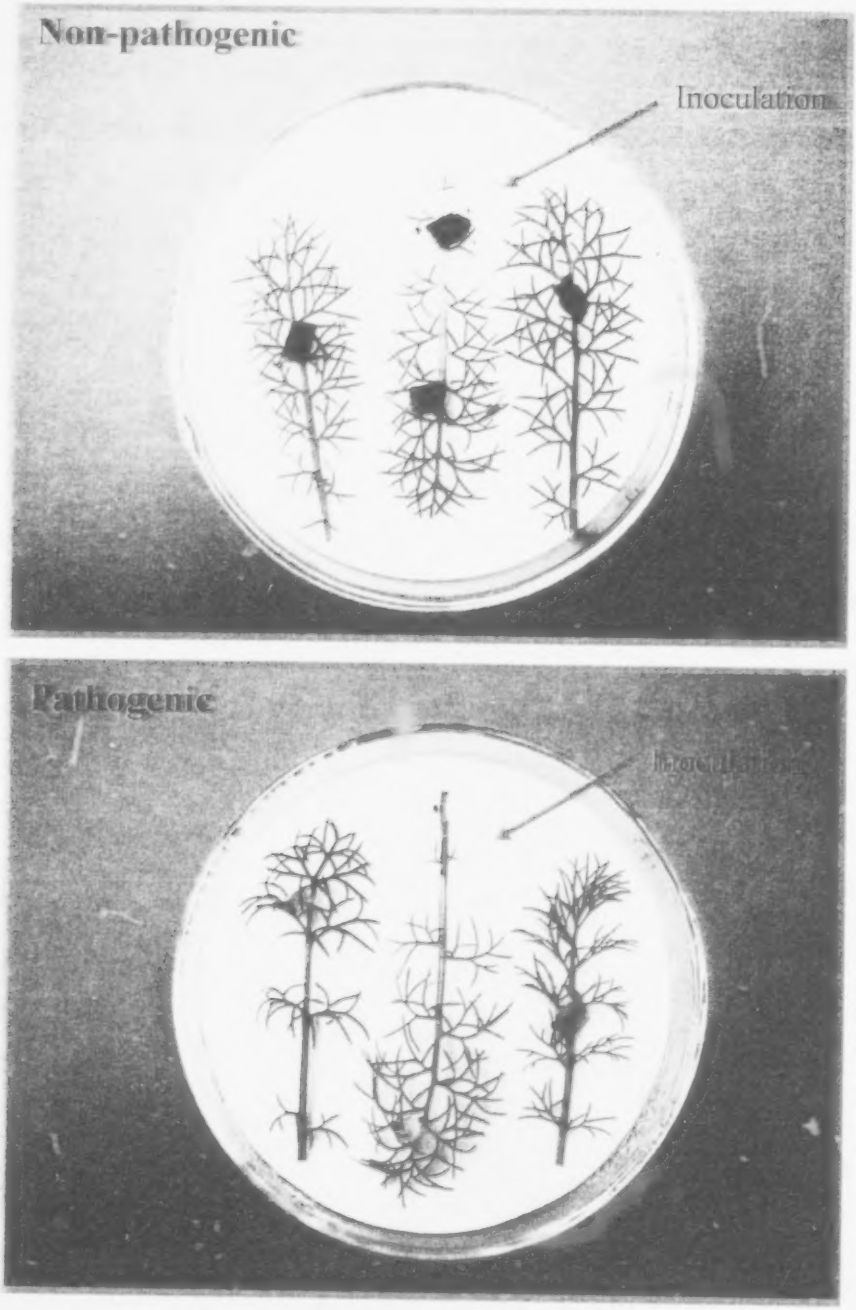


Photo 1. Bioassay for pathogenicity testing. The organisms in the top and bottom plates are considered non-pathogenic and pathogenic, respectively, according to the presence of disease symptoms originating from the inoculation.

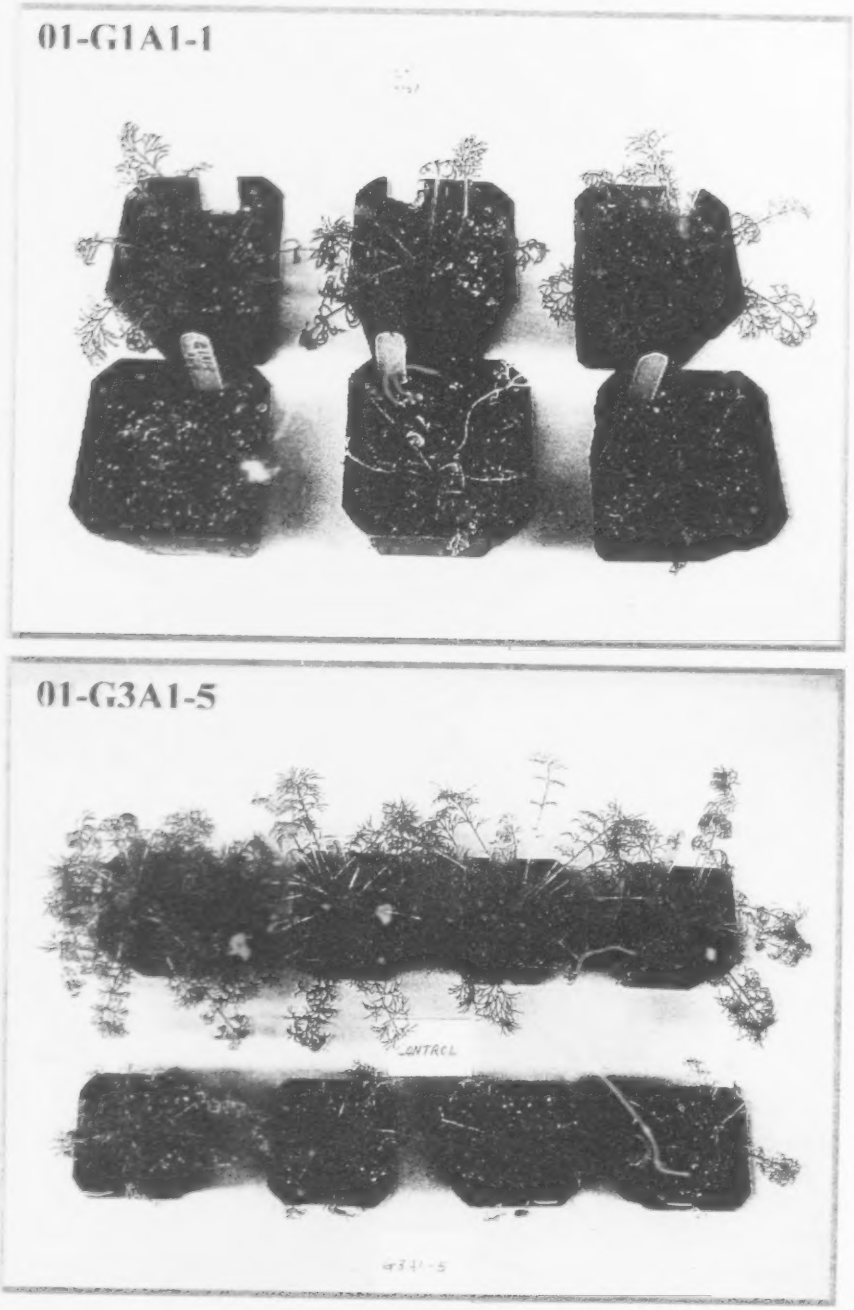


Photo 2. Weed-control efficacy by selected fungal pathogens (01-G1A1-1 and 01-G3A1-5) on the whole plant of scentless chamomile at 5-leaf (upper photo) and 12-leaf (lower photo) stages, respectively. Plants in the upper rows are untreated controls.

Photo 3. Effect of spore concentration of isolate 11FBr on control of scentless chamomile at whole-plant level. From the left to right, the concentration is 0, $5x10^4$, $5x10^5$, and 5×10^6 spores/ml, respectively.

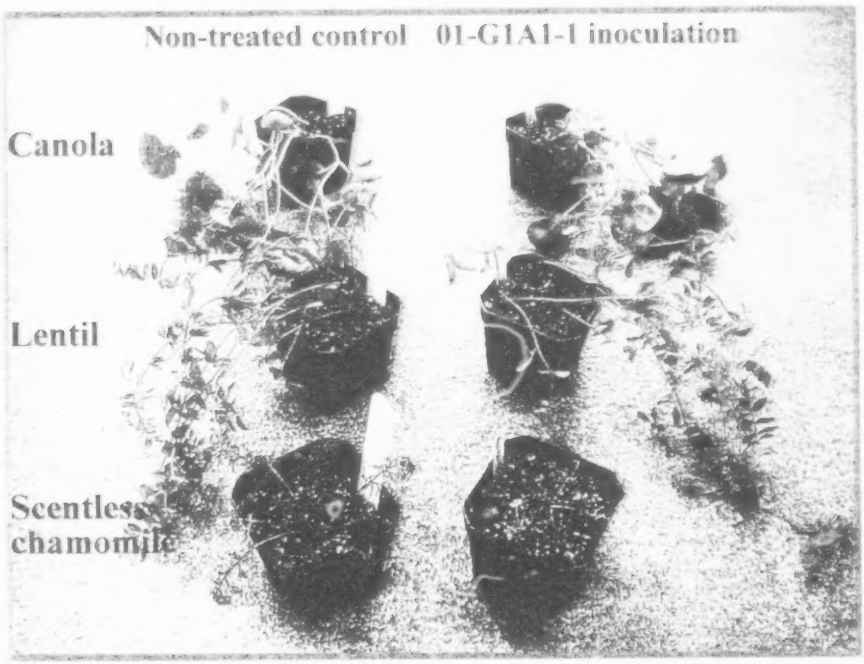
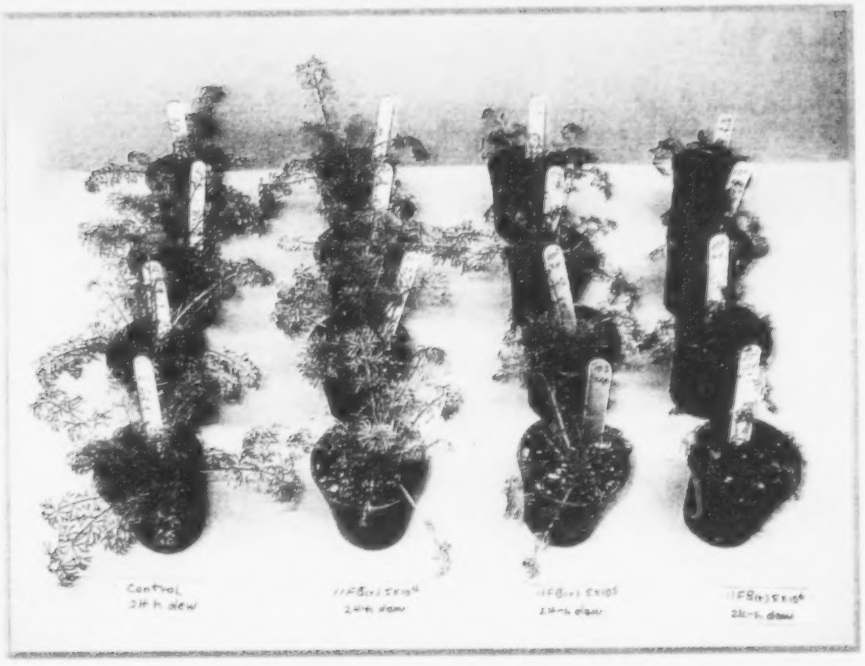


Photo 4. Different reactions by scentless chamomile, lentil, and canola to foliar inoculation using aqueous spore suspensions of isolates 01-G1A1-1 in a host-range test. This pathogen is host specific, causing no disease on the crop plants.



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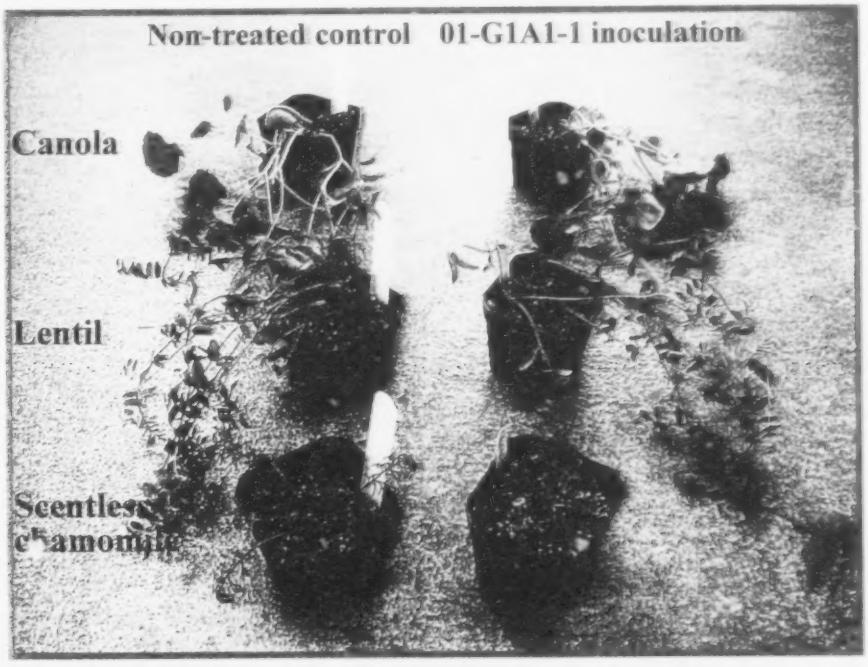

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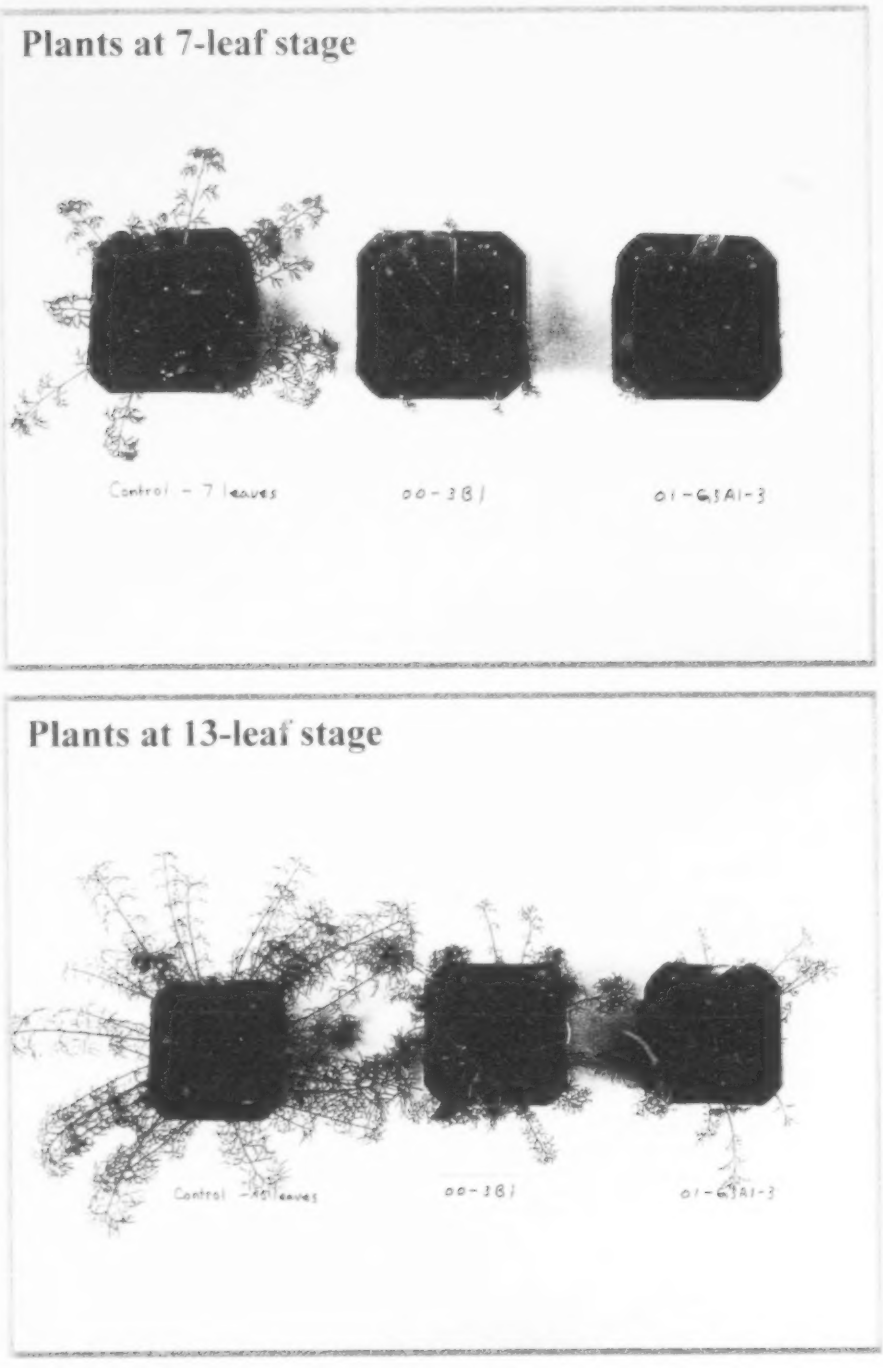

Photo 5. Comparison of weed-control efficacy of a foreign fungal isolate (01-G1A1-1) with an indigenous isolate (00-03B1) on scentless chamomile at 7-leaf (upper photo) and 13-leaf (lower photo) stages.

Appendix 3. Identification reports on *Colletotrichum* sp. isolates obtained from scentless chamomile in Europe and Saskatchewan.

Centraalbureau voor Schimmelcultures
Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW)

Agriculture and Agrifood Canada
Dr. Gary Peng
Saskatoon Research Center
107 Science Place
Saskatoon, Saskatchewan
Canada S7N OX2

Utrecht, October 29, 2002

IDENTIFICATION SERVICES

Your reference: letter of September 11, 2002 Our reference: det 251-2002

Dear Dr. Peng,

Herewith we inform you about the result of our identification of your 1 strain/sample.

Colletotrichum sp.

Morphologically, this strain is intermediate between *C. capsici* (Syd.) Butl. & Bisby, which is known to be pathogenic to plants from various families, and *C. circinans* (Berk.) Vogl., which is supposed to be specific to *Allium* spp. We suspect that further taxonomic work on these and other species with falcate conidia, viz., *C. phyllachoroides* (Ell. & Everh.) Arx, *C. corchori* Ikata & Tanaka, and *C. limicola* Pethybr. & Lafferty (= *C. lini* (Westerd.) Tochinai,), will show, that morphological species identifications are fairly unreliable in the so-called *capsici*-group. At this point we are unable to provide a certain species identification.

Your strains might be of interest for our culture collection and we will prepare these strains for inclusions. However, if you do not agree with the depositions of your strains please inform us at your earliest convenience. For information you can contact us by telephone −31 (0)302122600. Our email address is identification@cbs.knaw.nl. Please state always our reference number when you contact us

The invoice of these identifications is enclosed.

Yours sincerely,

Dr R.A.Samson
Head of Dept. Applied Research & Services

CABI BIOSCIENCE

IDENTIFICATION SERVICES

Report: H14/03/YC2

Your ref: Colletotrichum sp. - 00-3B1

Your number	IMI number	Identification and comments
00-3B1	390032	**Colletotrichum truncatum** (Schwein.) Andrus & W.D. Moore. Please refer to my previous email. *Colletotrichum truncatum* is one of the falcate-spored species, and is part of the *C. dematium* - *C. capsici* complex. The name is traditionally applied to legume collections, but very little is known of host specificity in this group. Collections identified as *C. truncatum* have rather short and wide conidia compared to other taxa in the *dematium* complex. In view of your biocontrol interests, you should be aware of the existence of *C. phyllachoroides* which is a poorly known species from *Artemisia* in the USA, of which no cultures are available. Judging from published reports, this has somewhat similar conidia to your strain. This isolate has been placed in the IMI Culture Collection. Report from Dr P.F. Cannon.

Agriculture and Agri-Food Canada
Agriculture et agroalimentaire Canada

National Program on Environmental Health, Biodiversity
Programme national sur la santé de l'environnement, Biodiversité

REQUEST FOR FUNGAL IDENTIFICATION - DEMANDE D'IDENTIFICATION FONGIQUE

ATTENTION :
We do not accept fungi known or suspected to be pathogenic to humans or animals.
Nous n'acceptons pas les champignons qui sont pathogènes, ou que l'on soupçonne de l'être, pour les humains ou les animaux.

ACKNOWLEDGEMENT of RECEIPT : This form will be returned to the client with the Lot Number.
ACCUSÉ DE RÉCEPTION : Ce formulaire sera retourné au client avec le numéro de l'envoi.

N.F.I.S. use only - Réservé à l'usage de S.N.I.F.
LOT NO. - N° DE L'ENVOI : 03M-25

CLIENT :
NAME - NOM: STÉPHAN BRIÈRE SIGNATURE AND DATE - SIGNATURE ET DATE:
ORGANIZATION - ORGANISATION: CFIA, PLANT QUARANTINE PESTS
ADDRESS - ADRESSE: 3851 FALLOWFIELD RD OTTAWA.
TEL - TÉL: FAX - TÉLÉCOPIEUR:
EMAIL - COURRIER ÉLECTRONIQUE:

Project - Projet : SASKATOON - BIOCONTROL FOR GREENHOUSE CHAMOMILE (SCENTLESS)
Laboratory File No. - N° de fiche du laboratoire :
Date required - Date requise :
Identify to - Identifier à : ☐ genus-genre ☐ species-espèce

NOTE: Your material may be deposited in the National Mycological Herbarium (DAOM) or in the Canadian Collection of Fungal Cultures (CCFC)
REMARQUE: Votre matériel peut être déposé à l'Herbier national de mycologie (DAOM) ou à la Collection de cultures fongiques canadiennes (CCFC)

☐ Check if you DO NOT WANT your material deposited in ether DAOM or CCFC.
☐ Cochez si vous NE VOULEZ PAS que votre matériel soit déposé au DAOM ou CCCF.
☐ Check if YOU WANT dried specimens returned.
☐ Cochez si VOUS VOULEZ qu'on retourne des spécimens séchés.

DESCRIPTION OF MATERIAL (Number of isolates, specimens, slides. Mark each appropriately with a number, host, medium, date isolated.)
DESCRIPTION DU MATÉRIEL (Nombre d'isolats, spécimens, lames. Inscrivez chaque échantillon avec le numéro, l'hôte, le milieu, la date d'isolement.)

isolates:
G9-H1 } Southern Germany
G11-H1 }
99-21A1 } Southern Saskatchewan.
331-31B3 }

REMARKS - OBSERVATIONS (Use separate sheet of paper if necessary - Utiliser une feuille séparée, si nécessaire)
(Tentative identification required; host/substrate, locality, collector, date of collection; additional data you think may aid the systematist in making identifications.)
(Identification provisoire obligatoire; hôte/substrat, endroit, collectionneur, date de collection; données supplémentaires qui, selon vous, peuvent aider le taxonomiste à effectuer les identifications.)

HOST: SCENTLESS CHAMOMILE - Matricaria perforata.
ORIGIN: SOUTHERN GERMANY & SOUTHERN SASKATCHEWAN
Tentative id: Colletotrichum truncatum (Schwein.) Andrus & E.D. Moore.

N.F.I.S. use only - Réservé à l'usage du S.N.I.F.
OUR MINIMUM ESTIMATE - NOTRE ESTIMATION MINIMUM : $ n/c

FEES: Hourly rate: $64.00/hr/scientist; $46.00/hr/technician
FRAIS : Taux horaire : $64.00/h/scientifique; $46.00/h/technicien(

Send to:
Elizabeth Fox, Research Technician
National Fungal Identification Service Manager
Biodiversity (Systematic Mycology and Botany)
Agriculture & Agri-Food Canada
960 Carling Ave., William Saunders Building (#49)
Central Experimental Farm (Maple Drive)
OTTAWA, ONTARIO, CANADA K1A 0C6

Envoyer à:
Elizabeth Fox, Technicienne de recherche
Gestionnaire du Service national d'identification fongique
Biodiversité (Systématique des champignons et des plantes)
Agriculture et Agroalimentaire Canada
960 avenue Carling, Édifice William Saunders (n° 49)
Ferme expérimentale centrale (Promenade Maple)
OTTAWA (ONTARIO) CANADA K1A 0C6

Specialist / Spécialiste: John Bissett
Completed / Complété:

Email - Courrier électronique : foxl@agr.gc.ca Tel - Tél: (613) 759-1375 Fax - Télécopieur : (613) 759-15

CABI BIOSCIENCE

IDENTIFICATION SERVICES

Report: H127/03/YC2
Your ref: Colletotrichum

Your number	IMI number	Identification and comments
00-193C	390737	Colletotrichum sp. All six of your strains belong to the same species, and identity with strain 00-3B1 is confirmed. There is little variation between the strains in morphological and cultural terms. Degree of melanization and sporulation appears to vary slightly between strains, but these differences are most unlikely to have any phylogenetic significance. Having the benefit of examining multiple strains of this species, I am now less confident that they belong to *Colletotrichum truncatum*. The conidia are similar in general terms to that species, but the *Matricaria* strains tend to have slightly straighter conidia with slightly more blunt ends. Biometric studies would be needed to confirm this distinction. ITS sequencing would be valuable in order to fix the identity of your species more accurately. I note that there are several sequences of *C. truncatum* in GENBANK, but there appears to be no independent confirmation of their identity. Other species such as *C. lini* which look similar to your fungus appear not to be sequenced. This isolate has not been placed in the IMI dried reference collection. Report from Dr P.F. Cannon.
01-40	390738	Colletotrichum sp. This isolate has not been placed in the IMI dried reference collection. Report from Dr P.F. Cannon.
01-60	390739	Colletotrichum sp. This isolate has not been placed in the IMI dried reference collection. Report from Dr P.F. Cannon.
02-G9 H1	390740	Colletotrichum sp. This isolate has not been placed in the IMI dried reference collection. Report from Dr P.F. Cannon.
02-G11 H1	390741	Colletotrichum sp. This isolate has not been placed in the IMI dried reference collection. Report from Dr P.F. Cannon.
00-3B1	390742	Colletotrichum sp. This isolate has not been placed in the IMI dried reference collection. Report from Dr P.F. Cannon.